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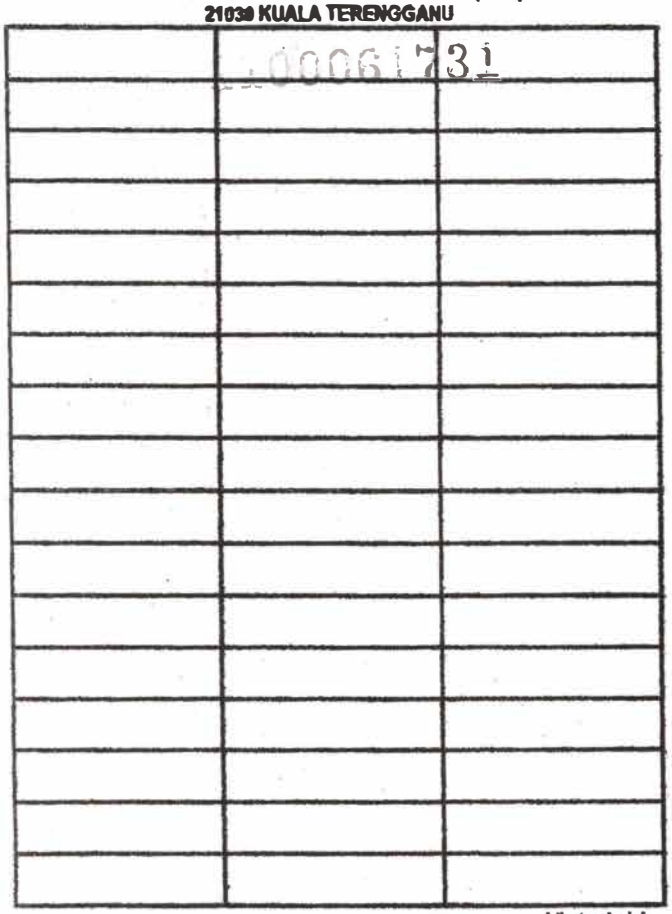

Lihat sebelah

DEVELOPMENT OF NESTED PCR PROTOCOL FOR THE ISOLATION OF PARTIAL GROWTH HORMONE GENE FRAGMENT IN MARBLE GOBY

NOOR SHAZANA BINTI MOHD SHAMLAN

This project report is submitted in partial fulfillment of requirement of the degree of Bachelor of Applied Science (Fisheries)

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ABSTRACT

A study was done to develop a Nested PCR protocol for the isolation of partial growth hormone (GH) gene fragment of Marble Goby. In this study, DNA from Marble Goby (*Oxyeleotris marmorata*) was extracted from the muscle tissue and used for PCR amplification. In the nested PCR protocol, amplification was done using two pairs of degenerate primers that were specific to the GH gene sequence. In this experiment, Nested PCR was used to confirm the PCR product as it allows discrimination between specific and nonspecific amplification signals. In this experiment, several optimizations were done. The optimizations involved four parameters that are annealing temperature, PCR cycle, concentration of Magnesium Chloride ($MgCl_2$) and polymerase enzymes. From the results obtained, the optimum annealing temperature was 32°C, the optimum PCR cycle was 35, the optimum $MgCl_2$ concentration was 2mM and the optimum enzymes used was Finnzymes DyNAzyme EXT DNA polymerase. PCR amplification using Fermentas Taq Polymerase (recombinant) and Finnzymes DyNAzyme EXT DNA polymerase produced four bands (250bp, 375bp, 500bp and 625bp) and eight bands (156bp, 250bp, 313bp, 375bp, 500bp, 625bp, 1000bp and 1118bp) respectively. Three fragments sizes 250bp, 375bp and 500bp have been isolated and purified. The study has succeeded in developing a nested PCR protocol for the isolation of partial GH gene fragment from Marble Goby. The development of the Nested PCR can serve as a useful method in obtaining a partial GH gene fragment for the development of GH gene probe towards the isolation of GH gene in fish.